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THE EFFECT OF BIOLOGICAL MEMBRANES TO FIBROBLAST PROLIFERATION: PLATELET-RICH FIBRIN RELEASATE VS AMNIOTIC (Research report)

Rezmelia Sari[1],Sri Pramestri Lastianny[1], Alya Nur Fadhilah[2]
[1]Department of Periodontics Faculty of Dentistry Universitas Gadjah Mada, Yogyakarta-Indonesia
[2]Faculty of Dentistry Universitas Gadjah Mada, Yogyakarta-Indonesia

ABSTRACT

Background: *Both amniotic and platelet-rich fibrin membranes are used to enhance open wound healing process clinically. The growth factors of these membranes are almost similar but the certain mechanism is still unclear especially in inducing fibroblast proliferation.* ***Objective:*** *to compare the effect of both membranes to fibroblast proliferation.* ***Methods:*** *This in vitro study was using fibroblast from periodontal ligament. The fibroblasts were cultured and then divided into 3 groups: fibroblasts with PRF releasate membrane (group I), fibroblasts only (group II), and fibroblasts with amniotic membranes (group III). The cells were observed in 24, 48 and 72 hours. Proliferation was tested by MTT assay and data was analyzed by two-way ANOVA followed by post-hoc test.* ***Results:*** *It showed that PRF releasate membrane induced fibroblast proliferation higher than amniotic in the first 24 hours meanwhile amniotic induced it two-fold in the next 24 hours. However, in the following time there were no significant differences between groups.* ***Conclusion:*** *It can be concluded that PRF membranes and amniotic membranes have the ability to accelerate proliferation of fibroblasts but have different effects at the time of induction.*

Keywords: *Amniotic, fibroblast; platelet-rich fibrin; proliferation; releasate*

Correspondence: *Rezmelia Sari, Department of Periodontics Faculty of Dentistry Universitas Gadjah Mada, Jl. Denta Sekip Utara Yogyakarta-Indonesia; rezmelia_sari@mail.ugm.ac.id*

INTRODUCTION

The healing process to form normal gingival contours of open wounds is relatively slow. Growth factor-containing membranes were applied to enhance the wound healing. Amniotic membrane is effective to cover the wound and to accelerate healing clinically. This membrane is easily obtained and triggers vascularity in the operating area but is fragile, thin and difficult to apply.[1]

Another biological membrane enriched with growth factors is platelet-rich fibrin (PRF) membrane.[2] Platelet-rich fibrin can be obtained from the patient's own blood, manipulated easily and relatively inexpensive.[2] In addition to membranes, the compression process also results releasate that rarely used in the clinic. Interestingly, it still contains growth factors[3] and gives good results to the gingival thickness after gingival recession treatment.[4]

Both membranes have been used post-periodontal surgery extensively. Clinical research has proved that amniotic membrane[1] and PRF membrane[5] can accelerate open wound healing but the mechanism is still unclear, especially in inducing the proliferation of fibroblasts. This current study was to compare the effect of both membranes to fibroblast proliferation.

Materials and Methods

The sample of this study was fibroblast cells obtained from the periodontal ligament of the second premolar extracted for orthodontic treatment. The amniotic membrane was produced by BATAN (research tissue bank) while membrane PRF releasate obtained from the blood

of a 37 years old male donor, clinically healthy, and willing to sign an informed consent. The procedure was approved by the Research Ethics Commission of the Faculty of Dentistry Universitas Gadjah Mada (UGM) Yogyakarta no 001543/KKEP/FKG-UGM/EC/2018. This study was conducted at the Dermatology and Venereology Laboratory of the Faculty of Medicine UGM.

Fibroblast cell subculture

The fibroblast cell medium in the flask was removed and 5 cc of sterile PBS was inserted into the flask and incubated for 10 minutes. After that PBS was removed and 1 cc of 0.25% Trypsin was added to the flask and incubated for 2-3 minutes. Cells are removed from the incubator and observed under a microscope. After the cell released, 5 cc of DMEM medium was added and the suspension was transferred into a 15 cc tube then centrifuged at 200 G for 10 minutes. The suspension was taken and the supernatant was removed. The suspension was steriled with 5cc PBS and put in 1cc of complete DMEM medium and centrifuged. Cultured fibroblasts were divided into 3 groups: fibroblasts incubated with PRF releasate membranes (group I), fibroblasts only (group II) and fibroblasts incubated with amniotic membranes (group III). The incubation was carried out for 24 hours (group A), 48 hours (group B), and 72 hours (group C).

The MTT assay

The medium was discarded and 200 ul of fresh medium were added followed by adding 50 ul MTT per well. The plates are wrapped in aluminum foil and incubated for 4-8 hours in a CO2 incubator at 37^{o}C. Supernatant is then discarded and 200 ul DMSO per well is added. The number of fibroblasts was read using a wavelength of 570 nm. The results were processed by software curve expert and analyzed by two-way ANOVA test followed by Post Hoc test.

A. Results

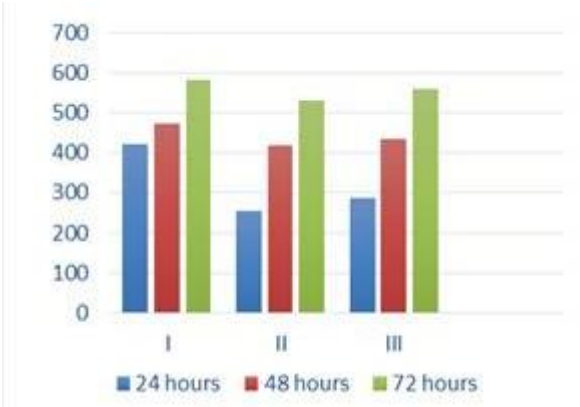

Figure 1. The number of fibroblast after incubated in Group I (PRF releasate membrane), Group II (fibroblast only) and Group III (amniotic membrane) at 24 hours , 48 hours , and 72 hours.

Figure 1 showed that the average number of fibroblast cells in all groups increased with incubation time. In the first 24 hours, group I had the highest number of fibroblast while group II had the lowest. After 24 hours incubation, the highest number of fibroblasts was found in the group with the addition of PRF releasate membranes while the lowest number was found in the control group. The same pattern was also found at longer incubation times of 48 to 72 hours. The results of the normality test with Kolmogorov-Smirnov showed that the data was normally distributed and homogeneous so continued with a Two-way ANOVA test and a Post hoc test (Table 1).

Table 1. The results of Post-hoc test in in Group I (PRF releasate membrane), Group II (fibroblast only) and Group III (amniotic membrane) after incubation

Duration of incubation	Studied groups	p value
24 hours	I-II	0.001*
	I-III	0.008*
	II-III	0.483
48 hours	I-II	0.265
	I-III	0.424
	II-III	0.746
72 hours	I-II	0.289
	I-III	0.662
	II-III	0.527

(*):significantly different

The post hoc test showed that the duration of incubation affected the number of fibroblast cells in each group ($p<0.05$). In the first 24 hours, there were significant differences in the number of

fibroblast cells in groups I, II and III ($p<0.05$). The highest number of fibroblast was found in group I folllowed by group III. Conversely, after 48 hours and 72 hours incubation, there were no significant differences in all test groups ($p>0.05$).

DISCUSSION

The process of wound healing is complex. This process consists of 4 phases namely homeostatic, inflammatory, proliferation, and maturation phase. In the proliferation phase, fibroblast cells play an important role because they are involved in several sequences especially epithelialization, fibroplasia, and contractions. The stage of fibroplasia is a process of fibroblast proliferation and fibrin migration.[6]

In this study, we found that both membrane can increase the fibroblast proliferation faster than control. The proliferation of fibroblast after the addition of PRF releasate membranes increased rapidly since the first 24 hours. The mean of fibroblasts after the addition of PRF releasate membranes is higher than the control or amniotic membrane. It might caused by the properties of PRF which progressively releases cytokines during fibrin matrix formation.[7] In addition to cytokines, PRF membranes contain Fibroblasts Growth Factor (FGF).

The FGF in PRF membranes can accelerate the proliferation of fibroblast.[9] This statement is in line with previous study which mentioned that platelet-rich fibrin from own blood of the individual can increase regeneration and acceerate the healing of the wound due to the consisting of various growth factor.[10]

The PRF membran is capable of increasing the adhesion and proliferations capacity of gingival fibroblasts. Thus, it is suitable for periodontal regeneration.[11]

The PRF membrane in this study was centrifuged at 3000 rpm for 10 minutes and compressed for 5 minutes. After that, the membrane was immersed into releasate and immediately added to fibroblasts as soon as possible. The aimed of this procedure was to maintain the growth factors contained in the membrane. This result was consistent with previous studies which proved that growth factors in PRF are influenced by platelet preparation, handling, and storage. PRF membrane should be used as soon as possible because of the optimal content of growth factors is at the beginning of preparation and still continues for 300 minutes later.[7]

The similar result was found in the amniotic membranes group. In this study, we can also found that fibroblast cell proliferation increases over the duration of incubation. It might caused by the properties of amniotic membrane as a biological scaffold for cell proliferation and differentiation.[12]

The amniotic membrane is a third generation membrane used for periodontal healing therapy. This membrane consists of a pluripotent stem cell which is a source for tissue transplantation.[13] Amnion has nonimmunogenic properties, to reduce inflammation, reduce tissue scarring, antibacterial, reduce pain in the applied body.[14]

The results of this study are also consistent with previous studies which state that PRF progressively produces cytokines during remodeling of the fibrin matrix in the healing process[15] while amnion induces proliferation of fibroblasts and vascular growth factor.[16]

Both membranes have a good effect in enhancing fibroblast proliferation compare to physiological processes. Interestingly, they have different pattern especially the onset of stimulation. PRF releasate membrane can stimulate proliferation in early 24 hours but amniotic membrane can duplicate the number of fibroblast in the next 24 hours. This difference is expected to be a matter of consideration for clinicians in choosing growth factors-containing materials to accelerate healing of open wounds.

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REFERENCES

1. Keerthi R, Vaibhav N, Raut R. Amniotic Membrane as a Biological Scaffold After Vestibuloplasty. J Maxillofac Oral Surg [Internet]. 2015;14(S1):383–7. Available from: http://link.springer.com/10.1007/s12663-014-0631-y

2. Bansal C, Bharti V. Evaluation of efficacy of autologous platelet - rich fibrin with demineralized - freeze dried bone allograft in the treatment of periodontal intrabony defects. 2013;17(3):361–6.

3. Marenzi G, Riccitiello F, Tia M, Di Lauro A, Sammartino G. Influence of leukocyte-

and platelet-rich fibrin (L-PRF) in the healing of simple postextraction sockets: A split-mouth study. Biomed Res Int. Hindawi Publishing Corporation; 2015;2015.

4. Karina VM, Lastianny SP NS. Pengaruh Penambahan Platelet Rich Fibrin Kompresi dengan dan Tanpa Releasate pada Bedah Mukogingival Perawatan Resesi Gingiva: Kajian Klinik. J Ked Gigi. 2016;320–6.

5. Sikkerimath BC, Dandagi S, Gudi SS JD. Comparison of vestibular sulcus depth in vestibuloplasty using standard Clark's technique with and without amnion as graft material. Ann Maxillofac Surg. 2012;2(1):30–5.

6. Kumar S, Kumar Gupta K, Bhowmick D, Singh A. Concepts of Healing in Periodontal Therapy-Part I. IOSR J Dent Med Sci e-ISSN. 2015;14(10):89–101.

7. Betancourt P, Elgueta R, Fuentes R. Treatment of endo-periodontal lesion using leukocyte-platelet-rich fibrin. A case report, Colomb Med, 2017:(48):204-207.

8. Choukroun, J. et al.. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology, 2006;101(3):56–60.

9. Tanya , Thomas BS. Platelet rich fibrin mebrane for recession coverage. J Dent. 2012; 2: 223-237

10. Kokdere NN, et al. The use of platelet-rich fibrin (PRF) and PRF-mixed particulated autogeneous bone graft in the treatment of bone defects; An experimental and histomorphometrical study. *Dental research journal* 12. 5(2015):418.

11. Bakhtiar H, Esmaeili S, Fakhr Tabatabayi S, Ellini MR, Nekoofar MH, Dummer PM. Second-generation platelet concentrate (Platelet-rich fibrin) as a scaffold in regenerative endodontics: A case series. *J. Endod.* 2017, 43,401-408

12. Su CY, Kuo YP, Tseng YH, Su CH, Burnouf T. In vitro release of growth factors from platelet-rich-fibrin firin (PRF): a proposal to optimize the clinical applications of PRF. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009; 44: 258-265

13. Tantin RD, Abbas B, Pandansari P, Hilmy N. Gingiva depigmentation using amniotic membrane. Human Amniotic Membrane. 2018; 307-318

14. Chopra A, Thomas B. Amniotic membrane: a novel material for regeneration and repair. Biomimetics Biomater Tissue Eng. 2013;18(1):1–8.

15. Padma R, Shilpa A, Kumar PA, Nagasri M, Kumar C, et al (2013). A split mouth randomized controlled study to evaluate the adjunctive effect of platelet-rich fibrin to coronally advanced flap in Miller's Class-I dan II recession defects. J Indian Soc Periodontol 17:631-636.

16. Fetterolf DE, Synder RJ. Scientific and clinical support for the use of dehydrated amniotic membrane in wound management. Wounds. 2012; 24: 299-309